Speciation analysis of trace elements in food and feed - status and future developments

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Speciation analysis of trace elements in food and feed - status and future developments

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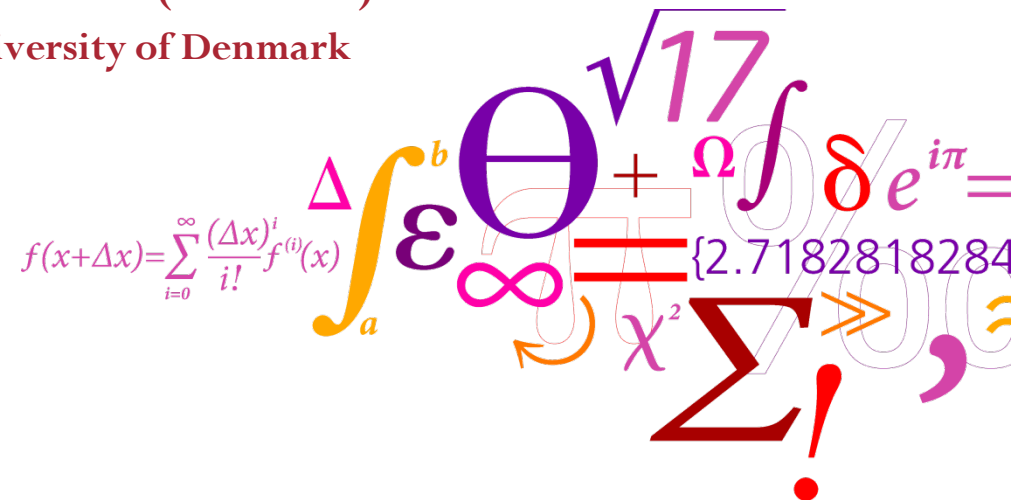


DTU Food
National Food Institute

AGENDA

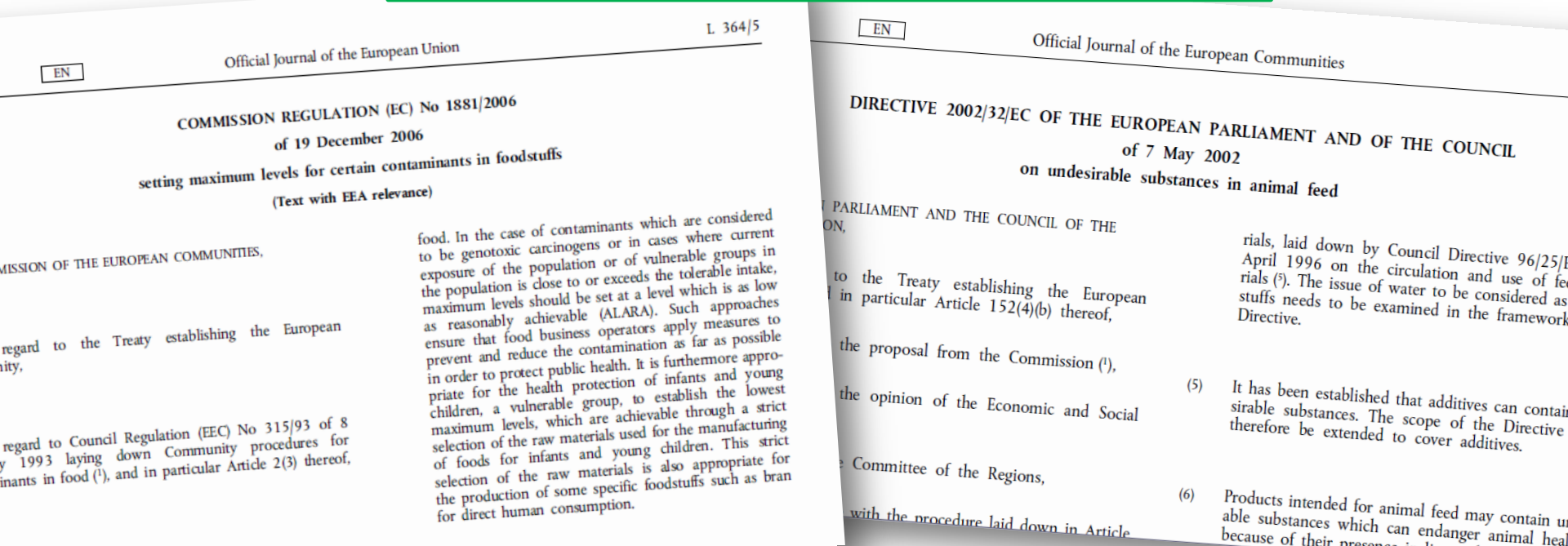
- **Speciation in relation to food and feed safety**
- **Selected examples**
  - 1. Arsenic speciation analysis (importance of inorganic arsenic)
  - 2. Organotin speciation analysis (food contact materials)
  - 3. Selenium speciation analysis (food fraud!)
  - .....and a little bit of nano!

CURRENT SITUATION IN EU LEGISLATION:

Foodstuffs
MLs for Pb, Cd, Hg and Sn
EU directive 2006/1881/EC

Animal feedingstuffs
MLs for As, Pb, Cd and Hg
EU directive 2002/32/EC

Only maximum levels for
TOTAL CONCENTRATION
of the metals



EN Official Journal of the European Union L 364/5

COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs
(Text with EEA relevance)

MISSION OF THE EUROPEAN COMMUNITIES,

regard to the Treaty establishing the European
nity,

regard to Council Regulation (EEC) No 315/93 of 8
y 1993 laying down Community procedures for
inants in food [1], and in particular Article 2(3) thereof,

food. In the case of contaminants which are considered to be genotoxic carcinogens or in cases where current exposure of the population or of vulnerable groups in the population is close to or exceeds the tolerable intake, maximum levels should be set at a level which is as low as reasonably achievable (ALARA). Such approaches ensure that food business operators apply measures to prevent and reduce the contamination as far as possible in order to protect public health. It is furthermore appropriate for the health protection of infants and young children, a vulnerable group, to establish the lowest maximum levels, which are achievable through a strict selection of the raw materials used for the manufacturing of foods for infants and young children. This strict selection of the raw materials is also appropriate for the production of some specific foodstuffs such as bran for direct human consumption.

EN Official Journal of the European Communities

DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 7 May 2002
on undesirable substances in animal feed

PARLIAMENT AND THE COUNCIL OF THE
ON,

to the Treaty establishing the European
in particular Article 152(4)(b) thereof,

the proposal from the Commission [1],

the opinion of the Economic and Social

Committee of the Regions,

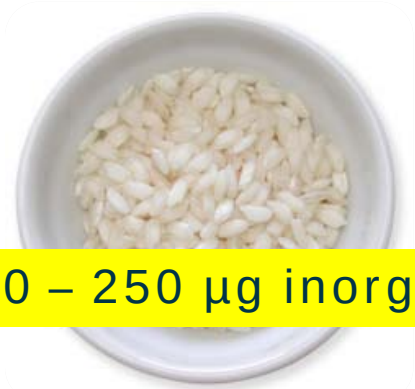
with the procedure laid down in Article

rials, laid down by Council Directive 96/25/E
April 1996 on the circulation and use of fe
rials [5]. The issue of water to be considered as
stuffs needs to be examined in the framework
Directive.

(5) It has been established that additives can contain
sirable substances. The scope of the Directive
therefore be extended to cover additives.

(6) Products intended for animal feed may contain u
able substances which can endanger animal hea
because of their

Example – arsenic speciation
Important for correct risk assessment



~ 40 – 250 µg inorg As

<10 µg inorg As

1 kg rice => 50-300 µg As

1 kg fish => 3000-10000 µg As

There is most focus on rice from a food safety point of view – why???

The chemical form of arsenic is important
=> Arsenic speciation

EFSA (2009) and JECFA (2010) opinions on arsenic in food

- Old PTWI value (WHO, 1988) was withdrawn *(15 µg/kg bw/week)*

efsa
European Food Safety Authority

- **NEW! $BMDL_{1.0}$ = 0.3 – 8 µg/kg bw per day for inorganic arsenic**
- *=> EU dietary exposures within this range for average and high level consumers*
- *=> Risk to some consumers cannot be excluded*

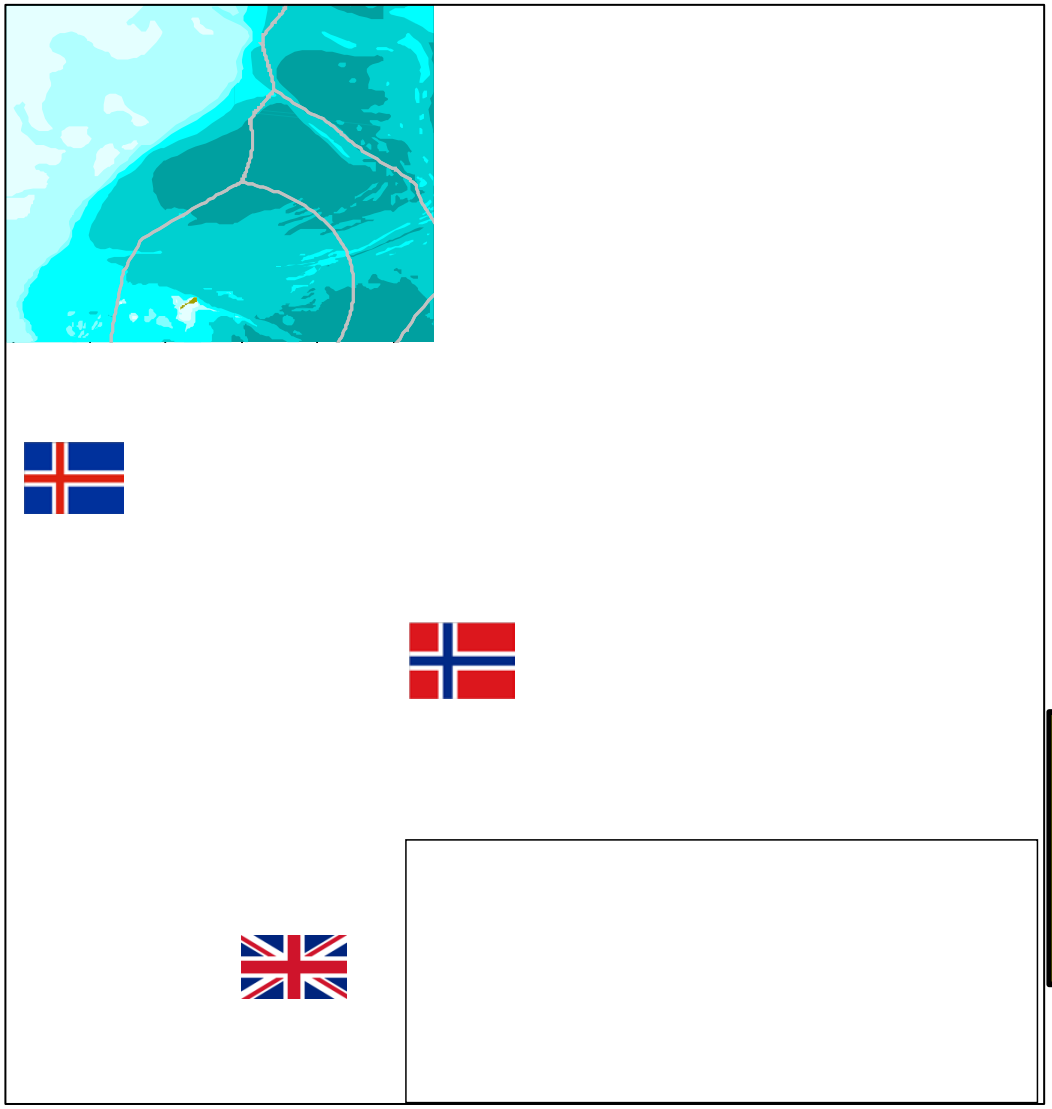
Food and Agriculture Organization of the United Nations

- **NEW! $BMDL_{0.5}$ = 3 µg/kg bw per day for inorganic arsenic**

  => 0.5% increased incidence of lung cancer for 12 y exposure

- *"…there is a need to produce speciation data for different food commodities to support dietary exposure assessment…"*
- *"…more accurate information on the inorganic arsenic content of foods is needed to improve assessments of dietary exposures to inorganic arsenic"*
- *"…need for validated methods for selective determination of inorganic arsenic in food matrices"*

Inorganic arsenic in wild caught fish => no concern



Norwegian survey

900 individual fish samples

- Atlantic halibut
- Cod
- Greenland halibut
- Mackerel
- Herring
- Tusk

Results

Total arsenic……..0.3-110 mg/kg

Inorganic arsenic….< 0.01 mg/kg

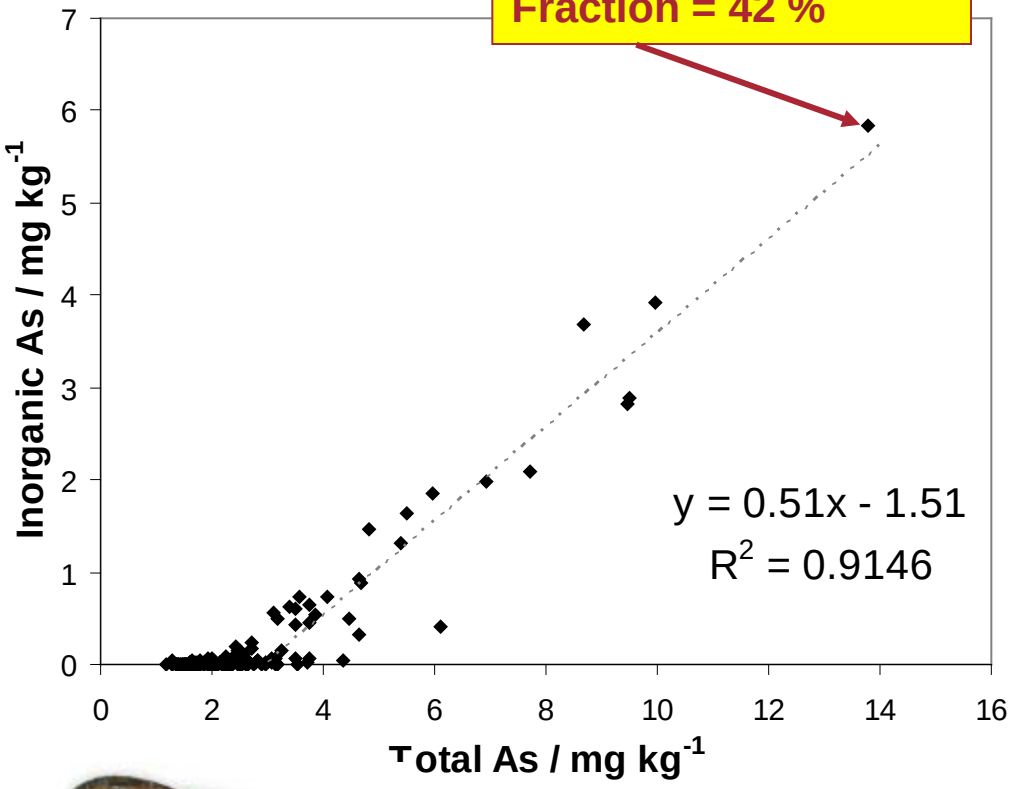
(only 37 samples > LOQ)

NIFES NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Julshamn and Sloth, 2011, in prep

...but in bivalves high contents in some samples...

Total As = 13.8 mg/kg
Inorg As = 5.8 mg/kg
Fraction = 42 %

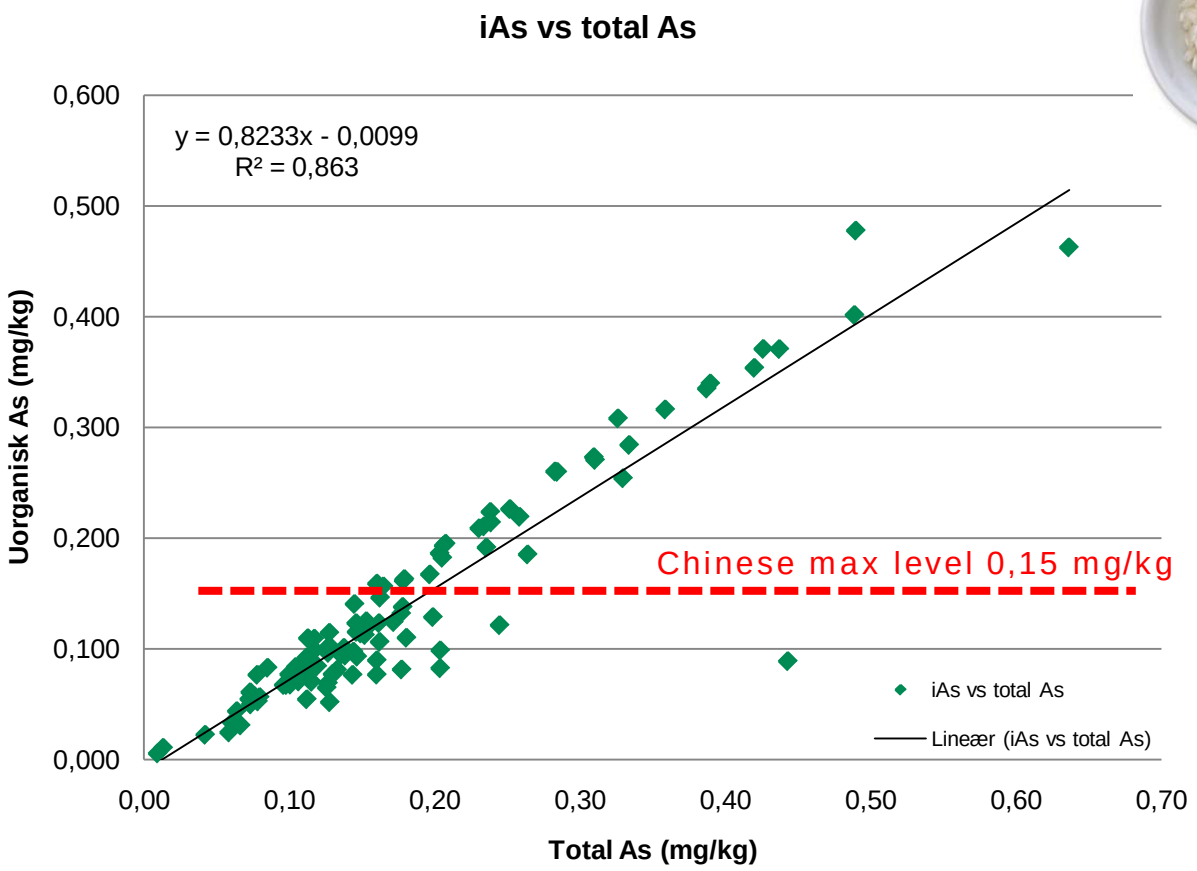


Data from 175 blue mussel (*Mytilus edulis*) samples collected along the Norwegian Coastline.

NIFES
NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Sloth and Julshamn, 2008, J. Agri.Food Chem., 56, 1269-1273

Arsenic in rice products DK - survey



105 samples

-white rice
-brown rice
-red rice
-black rice
-rice crackers

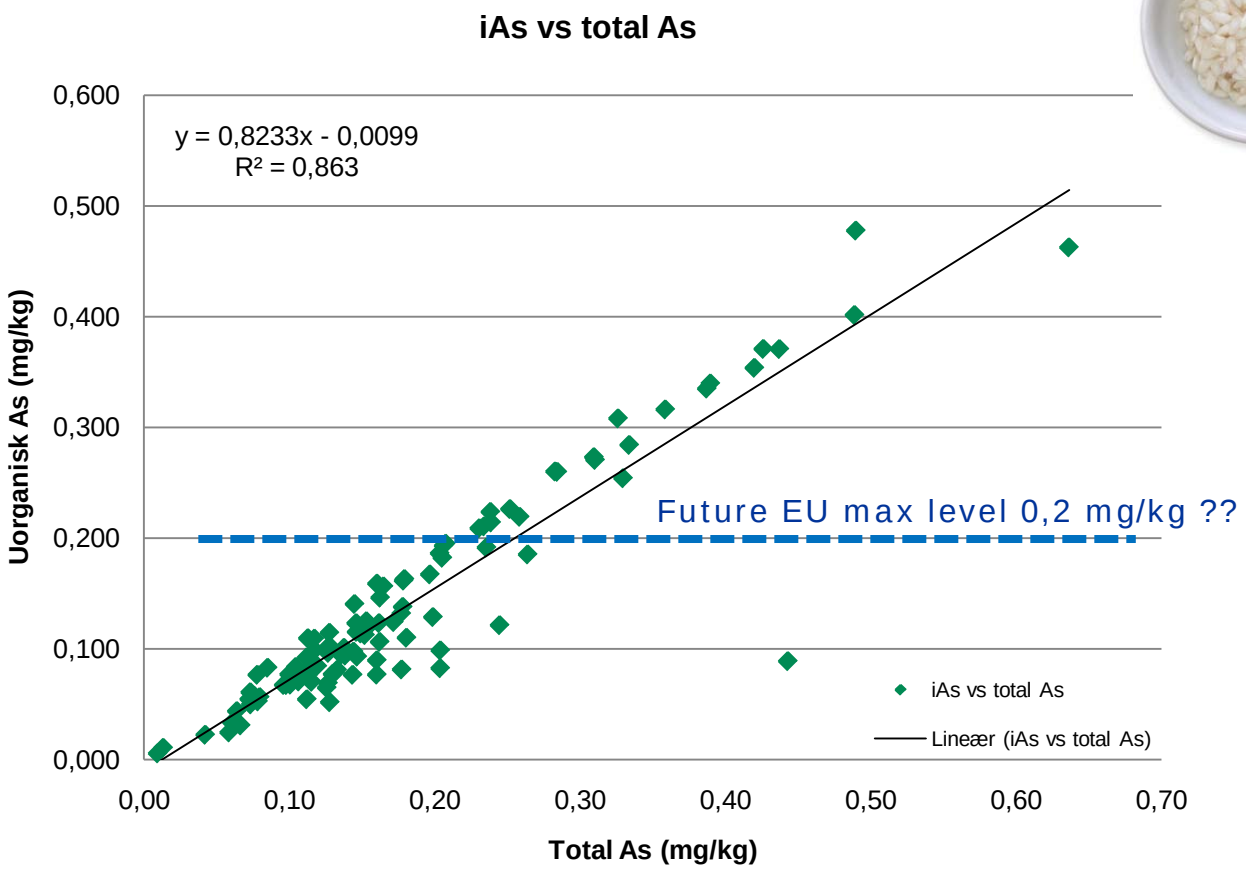
33 samples > 0,15 mg/kg

-2 parboiled (20%)
-4 brown (50%)
-4 red (50%)
-5 black (71%)
-1 Basmati (10%)
-1 Pudding rice (9%)
-1 wild rice (20%)
-15 rice crackers (100%)

Ministry of Food, Agriculture and Fisheries
Danish Veterinary and Food Administration

Sloth et al, in prep

Arsenic in rice products DK - survey



105 samples

-white rice
-brown rice
-red rice
-black rice
-rice crackers

22 samples > 0,2 mg/kg

-1 parboiled (10%)
-1 brown (12%)
-3 red (37%)
-2 black (28%)
-0 Basmati (0%)
-0 Pudding ri (0%)
-0 wild rice (
-15 rice crac

Rice cracker mean: 0.31 mg/kg – intake 50 g/dag => 15 µg iAs (~1 µg/kg b

> EFSA $BMDL_{01}$ 0.3-8 µg/kg bw/dag

Sloth et al, in prep

Inorganic arsenic in chinese food supplements

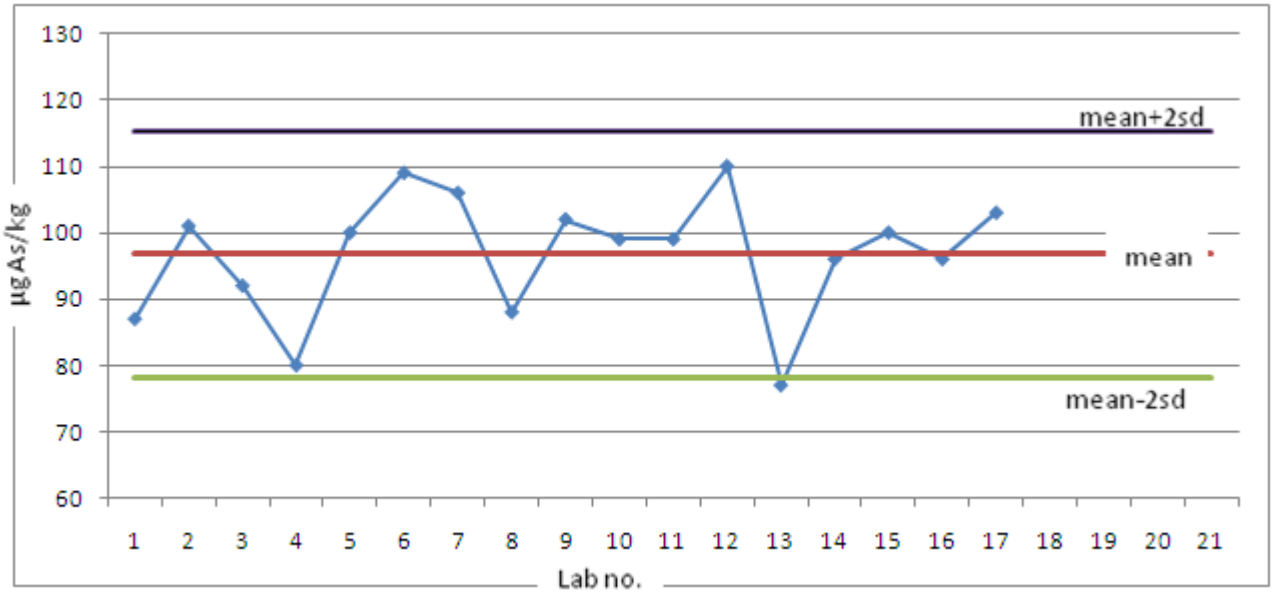
Name of Food supplement	Total Arsenic (μg/g)	Inorganic arsenic (μg/g)
Xiao Yao Wan	0.82	0.85
Bu Zhong Yi Qi Wan	0.62	0.50
Da Bu Yin Wan	0.59	0.55
Six Flavor teapills	0.72	N.D.
Golden Book Teapills	0.58	0.57
Xiang Sha Liu Jun Zi Wan	0.94	0.80
Gan Mao Ling	**1.24**	**1.01**
Chuan Xin Lian	5.00	3.17
Bi Yan Pian	0.70	0.58
Arouse power	1.12	1.02
Bio Chlorella	0.62	0.21
Unik Spirulina Kapsler	2.59	0.13
Chlorella	0.58	0.03
Ez-Biloba	0.63	0.67
Qvinde Dong Quai	0.68	0.48

Gan Mao Ling
(against flu and common cold)

Rec dose: 18 pills per day
⇒ iAs ~ 13 µg/day
⇒ 0.22 µg/kg bw/day (@60 kg)
Close to EFSA $BMDL_{01}$!!

Hedegaard and Sloth, in prep

Inorganic As in SRM NIST1586a – literature data



Mean = 0.097 mg/kg

	Extraction solution	Detection	iAs (µg/kg)			Reference
1	2M TFA	LC-ICPMS	87	+/-	9	Ackermann (2005)
2	Enzymatic, pepsin and pancreatin	LC-ICPMS	101	+/-	7	Ackermann (2
3	2M TFA	LC-ICPMS	92	+/-	2	Heitkemp
4	2M TFA	LC-ICPMS	80	+/-	16	Willia
5	2M TFA	LC-ICPMS	100	+/-	10	Willia
6	MeOH:H2O	LC-ICPMS	109	+/-	3	D'Ama
7	Enzymatic, alfa-amylase	LC-ICPMS	106	+/-	7	Kohlme
8	Enzymatic, protease and alfa-amylase	LC-ICPMS	88	+/-	6	Sanz (2005
9	1M H3PO4	HG-AFS	102	+/-	2	Matosreyes (2
10	1% HNO3	LC-ICPMS	99	+/-	4	Raab (2009)
11	1% HNO3	LC-ICPMS	99	+/-	-	Sun (2008)
12	1% HNO3	LC-ICPMS	110	+/-	10	Sun (2009)
13	0,5 M TFA	LC-ICPMS	77	+/-	-	Heitkemper (2009)
14	enzymatic, proteas and alfa-amylase	LC-ICPMS	96	+/-	9	Mar (2009)
15	2M TFA	LC-ICPMS	100	+/-	12	Meharg (2008)
16	water	LC-ICPMS	96	+/-	3	Narukawa (2008)
17	0,07M HCL and 10%H2O2	LC-ICPMS	103	+/-	15	DTU Food (2009)

Good agreement between labs and methods

Animal feedingstuffs

Commission directive 2009/114/EC (amendment)

Undesirable substances	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
(1)	(2)	(3)
'1. Arsenic (*) (**)	Feed materials with the exception of:	2
	— meal made from grass, from dried lucerne and from dried clover, and dried sugar beet pulp and dried molasses sugar beet pulp,	4
	— palm kernel expeller,	4 (***)
	— phosphates and calcareous marine algae,	10
	— calcium carbonate,	15
	— magnesium oxide,	20
	— feedingstuffs obtained from the processing of fish or other marine animals, including fish,	25 (***)
	— seaweed meal and feed materials derived from seaweed,	40 (***)
	Iron particles used as tracer.	50
	Additives belonging to the functional group of compounds of trace elements except:	30
	— zinc oxide, manganese oxide and copper oxide,	100
	Complete feedingstuffs with the exception of:	2
	— complete feedingstuffs for fish and complete feedingstuffs for fur animals,	10 (***)
	Complementary feedingstuffs with the exception of:	4
	— mineral feedingstuffs,	12

Only max levels for total arsenic

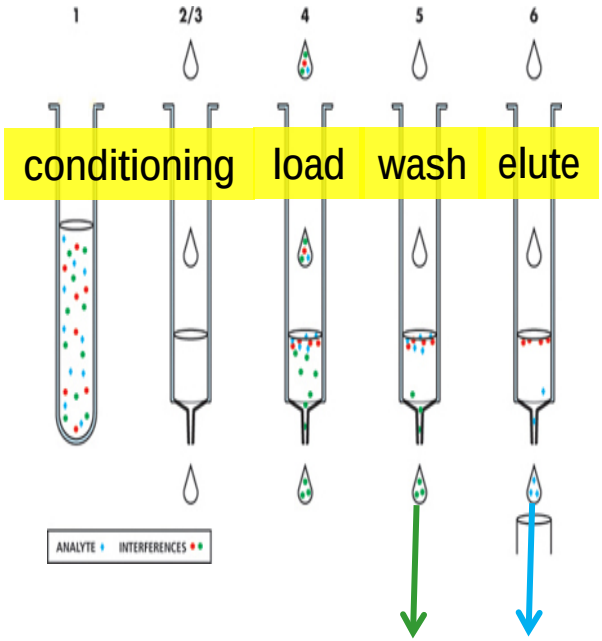
FOOTNOTE

(***) Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of **Inorganic arsenic lower than 2 ppm** is of particular importance for the seaweed species *Hizikia fusiforme*.'

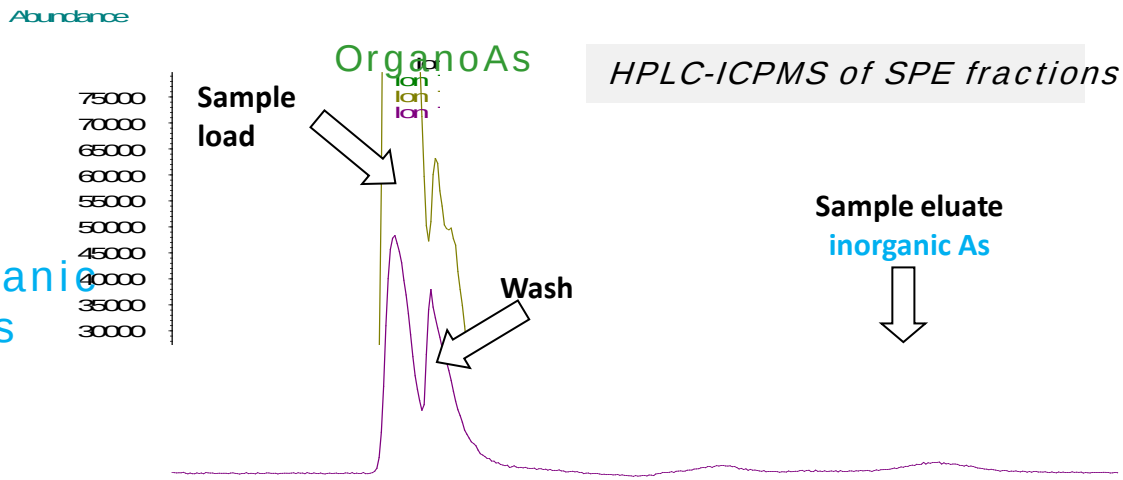
Speciation analysis is required !!

SPE-HG-AAS – a speciation alternative…

μ-wave extraction → Separation by SPE → Detection by HG-AAS



Sequential elution for selective off-line separation of inorg As from organo As species by SPE



HPLC-ICPMS of SPE fractions

Rasmussen & Sloth, in prep

SPE-HG-AAS

In-house validation data

Parameter	Result
Analysis time	2 x 7 h for 24 samples
LoD (mg/kg)	0.08
LoQ (mg/kg)	0.16
Repeatability (%RSD)	3 - 7
Accuracy (%)	90 - 104

Collaborative trial in marine feed material

- 10 labs provided results
- Method working range tested: 0.1 – 2.6 mg Kg^{-1}
- HorRat values <2 in the working range tested
- The method is fit for purpose
- Final publication as EN expected July 2012

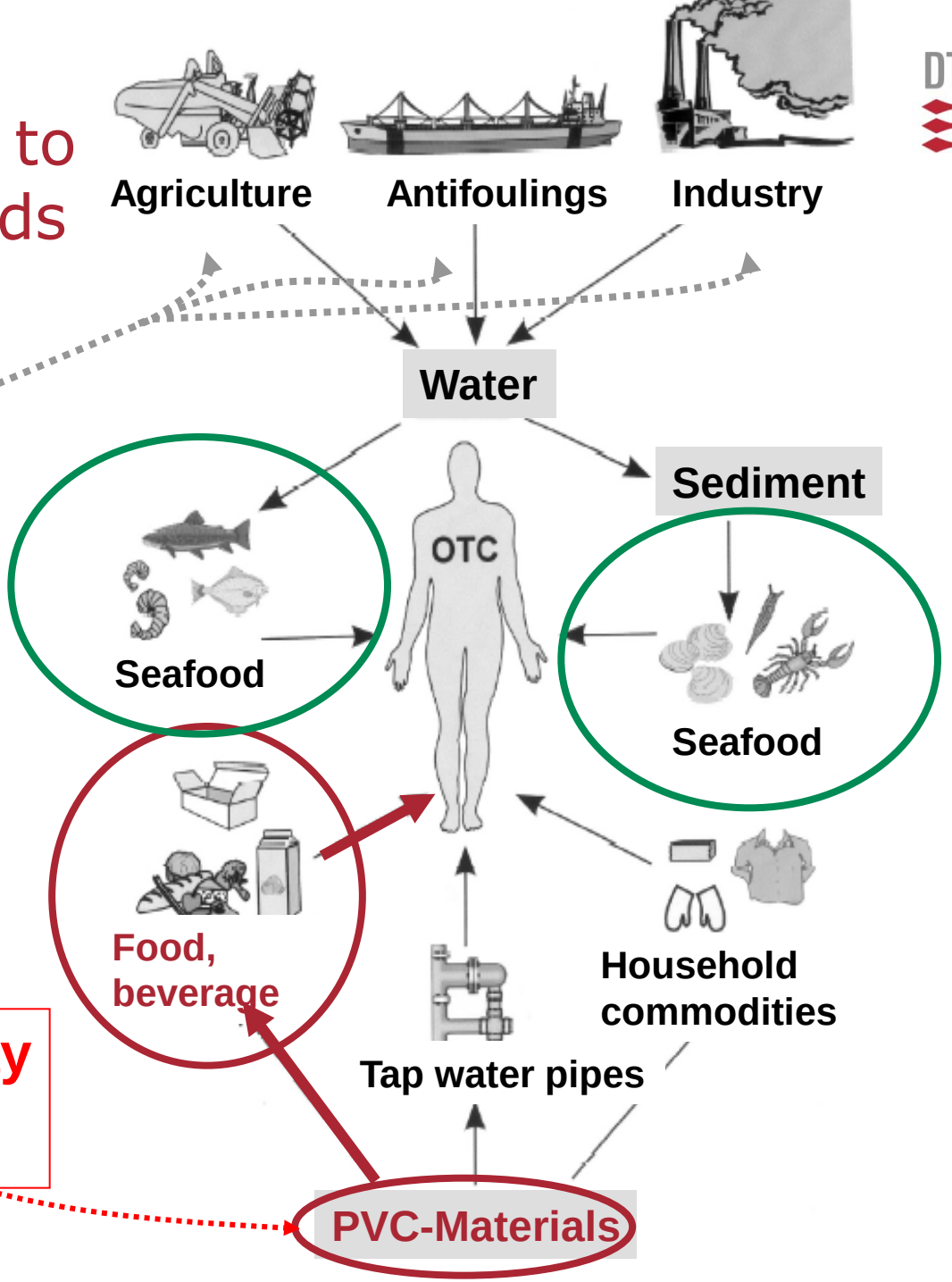
Routes of exposure to organotin compounds

Used in
- **Agriculture**
- **Antifoulings**
- **Industry**

- **PVC-Materials**

TDI: 0.25 µg/kg bw/day

∑ TBT, DBT, TPhT and DOT



Hoch, Appl. Geochem., 2001, 719-43

Legislation on OTCs in Food Contact Materials

Compounds	Maximum level (µg Sn/kg foodstuff)
∑ DBT, TBT, TPhT and DOT	40 (6)
∑ MMT, DMT	180
MOT	1200
MDDT	12000 (50)
DDDT	24000 (50)

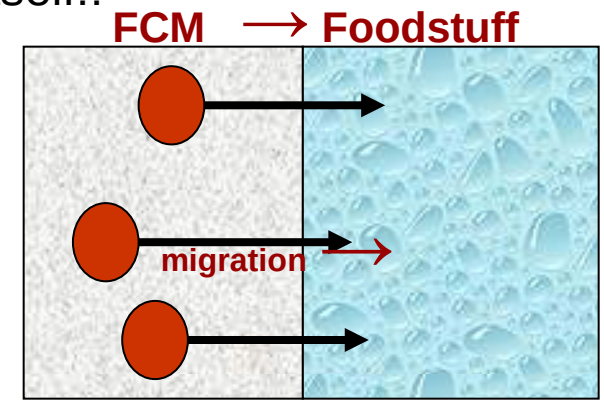
Ref: EFSA (2005); proposed EFSA values in parenthesis

Assumptions:

- 1 kg food per 6 dm^2

- 100 mL in contact with 0.6 dm^2

- Max levels on organotin migrating from the packaging material
- Testing by the use of food simulators (water, acid, oil, alcohol etc)
- BUT no maximum levels on organotins in the foodstuff itself!!

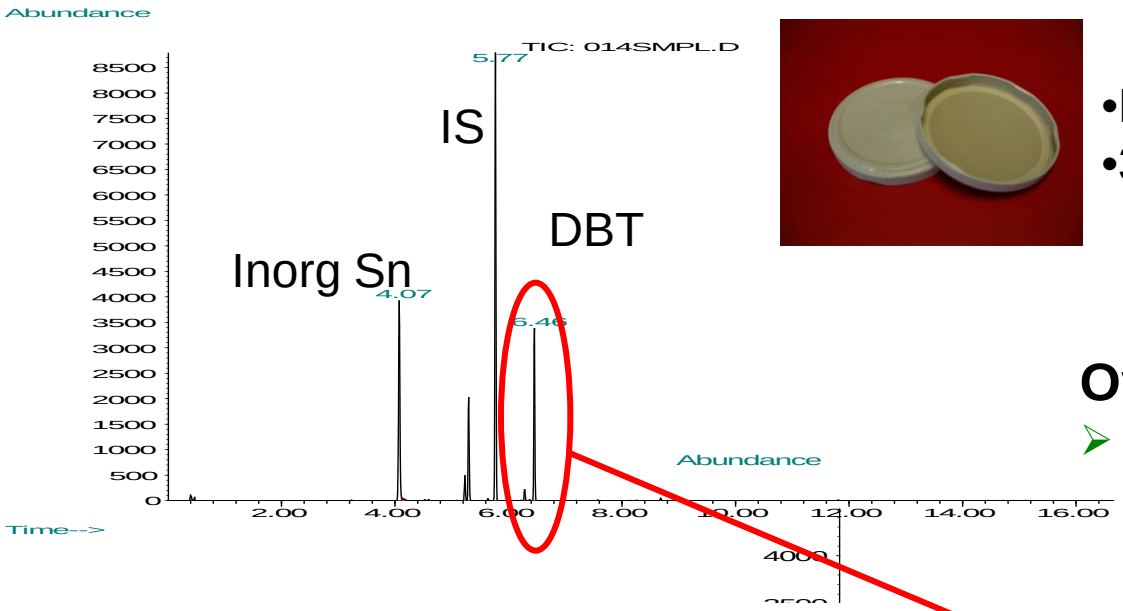


Organotin migration from Food Contact Materials II

Small scale survey on 33 FCMs

Baking paper, PVC cling films, silicone baking forms, lids with PVC gaskets

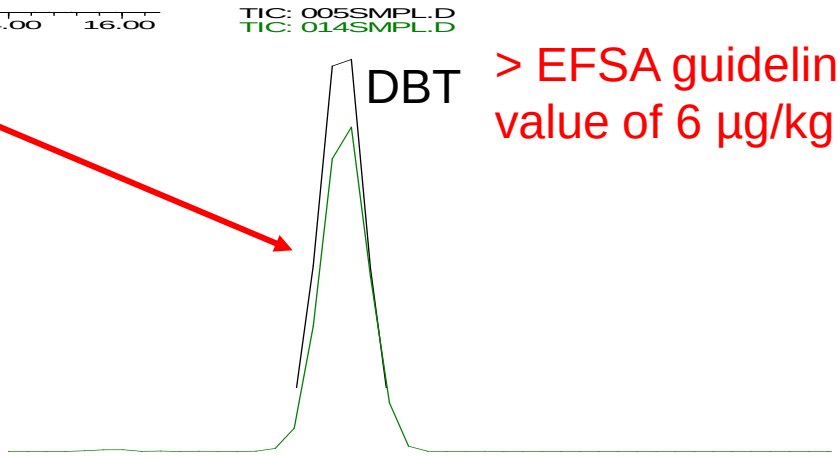
PUR-agglomerated cork wine stoppers



- PVC lid
- 3% acetic acid

Overlaid standard and sample

➢ DBT concentration: 9.9 µg/kg



> EFSA guideline value of 6 µg/kg

Output of DK survey:
-33 samples
-11 contained OT (mainly DBT)
-3 exceeded EFSA guideline limit

Sloth et al, in prep

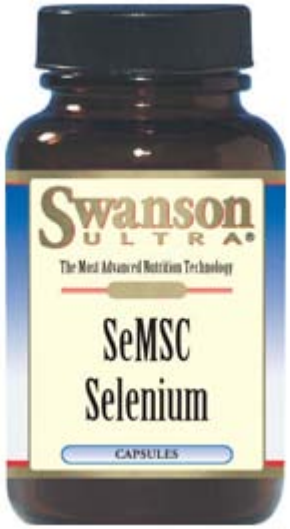
Selenium in commercial food supplements

Organic bound Se?

Se yeast?

Selenomethionine?

Selenite?



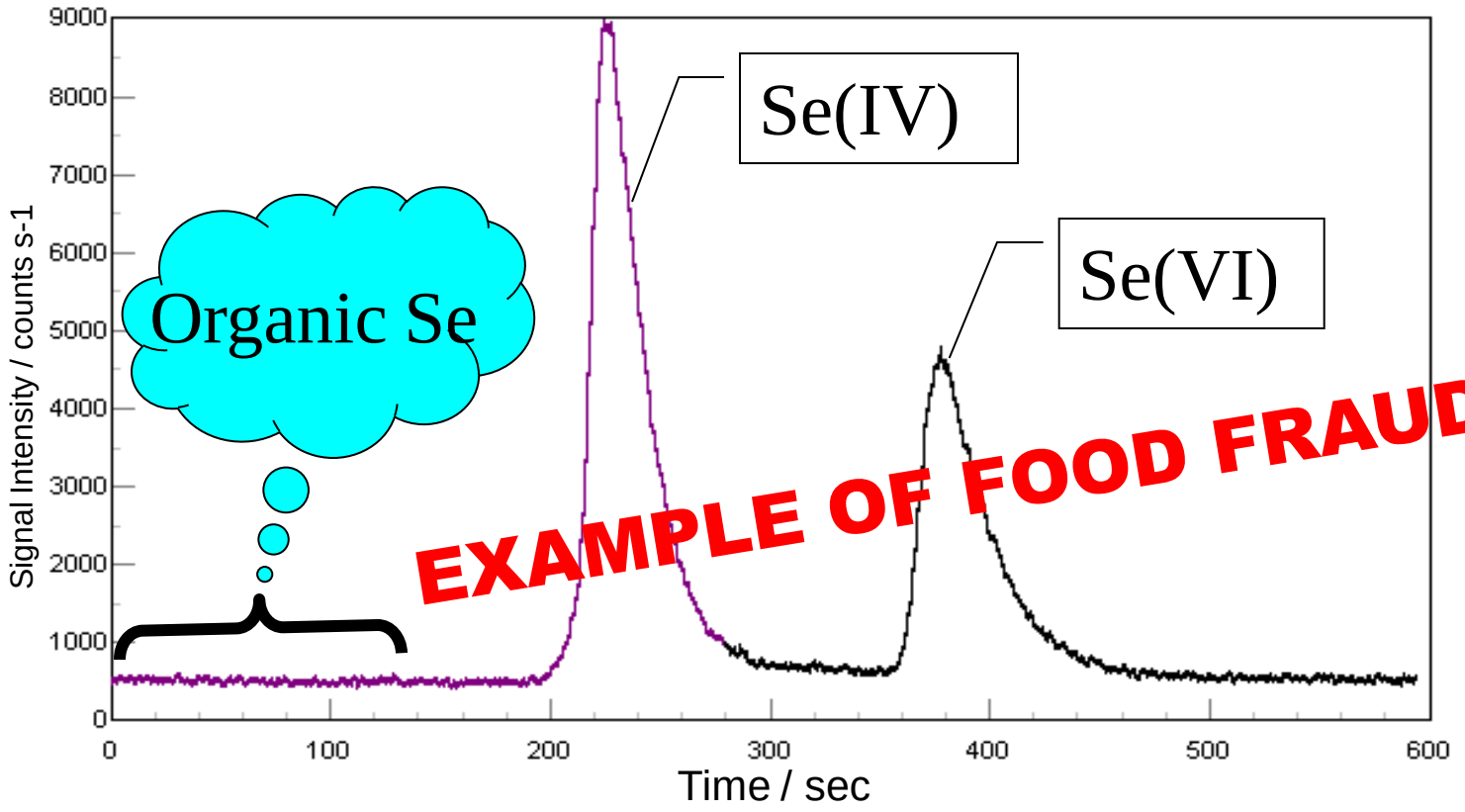
Amino chelated Se ?

Selenate?

Is the selenium source declared correctly??

Se speciation by HPLC-ICPMS

Supplement declared as *organic bound* Selenium (125 µg/tablet)



Larsen et al, unpublished data

Speciation summary

- ✓ speciation methods are more and more commonly used
- ✓ instrumentation is widely available
- ✓ legislation on species has started
- ✓ …and more is expected in the future!

- ✓ standardised methods are not ready!
- ✓ …but the need is known by authorities
- ✓ legislation is still behind!
- ✓ Lack of CRMs (e.g. for iAs)

Perspective – trace elements in nanoform

NanoLyse Project "Nanoparticles in Food: Analytical methods for detection and characterisation"

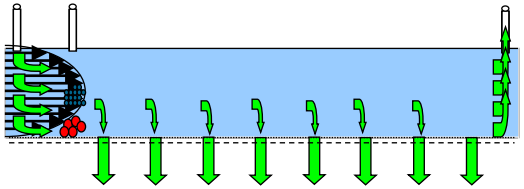
Validated methods for the determination of inorganic ENP in food extracts, based on size separation, size determination and specific detection

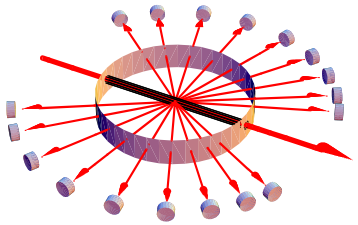
silver nanoparticles in lean meat

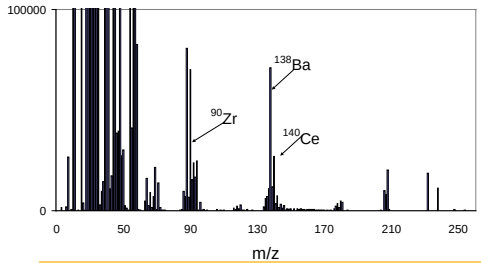
silica nanoparticles in tomato soup

http://www.nanolyse.eu

DTU Food Analytical platform: AF4-MALS/DLS-ICP-MS







asymmetric flow field flow fractionation

(AF4)

optical detection multi angle (MALS) and dynamic light scattering (DLS), UV-vis absorption and fluorescence

inductively coupled plasma mass spectrometry

(ICP-MS)

particle separation according to their size (1nm – few µm)

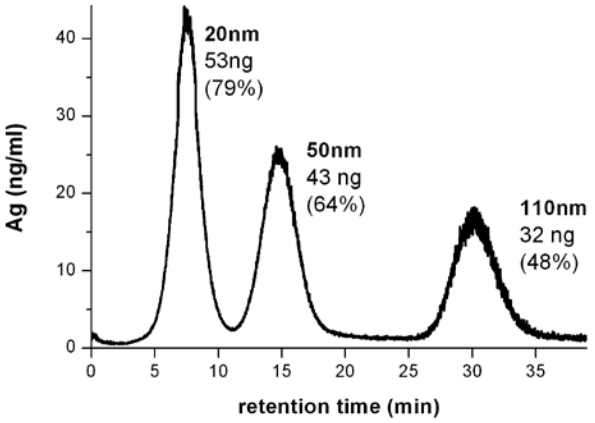
particle detection

size determination

elemental detection chemical identity

quantification

Fractogram =>



www.nanolyse.eu

Schmidt et al, Anal Chem, 83, 2461-2468 (2011)

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125th AOAC ANNUAL MEETING & EXPOSITION

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Thanks for your attention!